



EFSA CEF Panel (Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids , 2013. Scientific Opinion on Flavouring Group Evaluation 2 07 (FGE.2 07)

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 207 (FGE.207):

Consideration of genotoxic potential for one branched-chain aliphatic acyclic α,β -unsaturated 2-alkylated aldehyde with additional double-bonds, from subgroup 1.1.2 of FGE.19 and four alicyclic aldehydes with the α,β -unsaturation in a side-chain, from subgroup 2.1 of FGE.19, which are considered to be covered by the one substance of subgroup 1.1.2, by EFSA¹

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)^{2,3}

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ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate the genotoxic potential of one flavouring substance, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], from subgroup 1.1.2 of FGE.19, which is considered to be representative for four substances, 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712], from subgroup 2.1 of FGE.19. The Flavour Industry has provided genotoxicity studies for the representative substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] and these data are considered by EFSA to be representative for the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712]. Based on the new data, the Panel concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] from FGE.19 subgroup 1.1.2 does not give rise to concern with respect to genotoxicity and can accordingly be evaluated using the Procedure. This conclusion can also be applied to the four substances 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712] from FGE.19 subgroup 2.1 for which 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] is representative.

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KEY WORDS

FGE.207, α,β -unsaturated alicyclic aldehydes, α,β -unsaturated, α,β -unsaturated in side-chain, Subgroup 1.1.2, Subgroup 2.1, FGE.19

¹ On request from the European Commission, Question No EFSA-Q-2013-00024, EFSA-Q-2013-00025, EFSA-Q-2013-00026, EFSA-Q-2013-00027, EFSA-Q-2013-00028, adopted on 15 May 2013.

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SUMMARY

Following a request from the European Commission the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel) was asked to deliver a scientific opinion on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member states. In particular, the Panel was asked to evaluate flavouring substances using the Procedure as referred to in the Commission Regulation (EC) No 1565/2000.

The present Flavouring Group Evaluation 207 (FGE.207), corresponding to subgroup 2.1 of FGE.19, concerns the evaluation of genotoxicity data submitted on one α,β -unsaturated flavouring substance, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], from subgroup 1.1.2 of FGE.19 (FGE.201), which is considered to be representative for four substances, 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712], from subgroup 2.1 of FGE.19.

The α,β -unsaturated carbonyl structure is a structural alert for genotoxicity and the data on genotoxicity previously available for these FGE.19 subgroup 2.1 substances or structurally related flavouring substances did not rule out the concern for genotoxicity.

The Flavour Industry has provided new genotoxicity data for 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] from subgroup 1.1.2 of FGE.19 (FGE.201), requested in FGE.201. These data are considered by the Panel to be representative for the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from FGE.19 subgroup 2.1 due to the fact that all five substances have the α,β -unsaturation in an aliphatic side-chain and are methylated in the α -position of the α,β -unsaturation.

Based on the new data, the Panel concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] does not give rise to concern with respect to genotoxicity and accordingly it can be evaluated through the Procedure. This conclusion can also be applied to the four substances 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034] and santalyl phenylacetate [FL-no: 09.712] from FGE.19 subgroup 2.1 for which 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] is representative.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008 (EC, 2008) on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012 (EC, 2012). The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000 (EC, 2000).

EFSA has evaluated 11 flavouring substances, which correspond to subgroup 1.1.2 of FGE.19, in its evaluation of the flavouring group 201 (FGE.201). The opinion was adopted on 25 September 2008.

EFSA concluded that a genotoxic potential of the 11 α,β -unsaturated aldehydes and alcohol and related esters in the present FGE.201 could not be ruled out.

Information on one representative material 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] has now been submitted by the European Flavour Association. This information is intended to cover also the re-evaluation of the following four substances from FGR.19 subgroup 2.1 (FGE.207):

- 12-beta-Santalen-14-ol [FL-no: 02.216]
- 12-alpha-Santalen-14-ol [FL-no: 02.217]
- Santalyl acetate [FL-no: 09.034]
- Santalyl phenylacetate [FL-no: 09.712]

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substances.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following five substances: 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034], santalyl phenylacetate [FL-no: 09.712] and 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], in accordance with Commission Regulation (EC) No 1565/2000.

ASSESSMENT

1. History

Flavouring Group Evaluation 19 (FGE.19) contains 360 flavouring substances from the EU Register being α,β -unsaturated aldehydes or ketones and precursors which could give rise to such carbonyl substances via hydrolysis and / or oxidation (EFSA, 2008a).

The α,β -unsaturated aldehyde and ketone structures are structural alerts for genotoxicity. The Panel noted that there were limited genotoxicity data on these flavouring substances but that positive genotoxicity studies were identified for some substances in the group.

The α,β -unsaturated carbonyls were subdivided into subgroups on the basis of structural similarity (EFSA, 2008a). In an attempt to decide which of the substances could go through the Procedure, a (quantitative) structure-activity relationship ((Q)SAR) prediction of the genotoxicity of these

substances was undertaken considering a number of models (DEREKfW, TOPKAT, DTU-NFI-MultiCASE Models and ISS-Local Models, (Gry et al., 2007)).

The Panel noted that for most of these models internal and external validation has been performed but considered that the outcome of these validations was not always extensive enough to appreciate the validity of the predictions of these models for these α,β -unsaturated carbonyls. Therefore, the Panel considered it inappropriate to totally rely on (Q)SAR predictions at this point in time and decided not to take substances through the Procedure based on negative (Q)SAR predictions only.

The Panel took note of the (Q)SAR predictions by using two ISS Local Models (Benigni and Netzeva, 2007a; Benigni and Netzeva, 2007b) and four DTU-NFI MultiCASE Models (Gry et al., 2007; Nikolov et al., 2007) and the fact that there are available data on genotoxicity, *in vitro* and *in vivo*, as well as data on carcinogenicity for several substances. Based on these data the Panel decided that 15 subgroups (1.1.1, 1.2.1, 1.2.2, 1.2.3, 2.1, 2.2, 2.3, 2.5, 3.2, 4.3, 4.5, 4.6, 5.1, 5.2 and 5.3) (EFSA, 2008a) could not be evaluated through the Procedure due to concern with respect to genotoxicity. Corresponding to these subgroups, 15 Flavouring Group Evaluations (FGEs) were established, FGE.200, 204, 205, 206, 207, 208, 209, 211, 215, 219, 221, 222, 223, 224 and 225).

For 11 subgroups the Panel decided, based on the available genotoxicity data and (Q)SAR predictions, that a further scrutiny of the data should take place before requesting additional data from the Flavouring Industry on genotoxicity. These subgroups were evaluated in FGE.201, 202, 203, 210, 212, 213, 214, 216, 217, 218 and 220. For the substances in FGE.202, 214 and 218 it was concluded that a genotoxic potential could be ruled out and accordingly these substances will be evaluated using the Procedure. For all or some of the substances in the remaining FGEs, FGE.201, 203, 210, 212, 213, 216, 217 and 220, the genotoxic potential could not be ruled out.

To ease the data retrieval of the large number of structurally related α,β -unsaturated substances in the different subgroups for which additional data are requested, EFSA has worked out a list of representative substances for each subgroup (EFSA, 2008b). Likewise an EFSA genotoxicity expert group has worked out a test strategy to be followed in the data retrieval for these substances (EFSA, 2008c).

The Flavouring Industry has been requested to submit additional genotoxicity data according to the list of representative substances and test strategy for each subgroup.

The Flavouring Industry has now submitted additional data and the present FGE concerns the evaluation of some of these data requested on genotoxicity.

2. Presentation of the Substances in the Flavouring Group 207

2.1. Description

The present Flavouring Group Evaluation 207 (FGE.207), corresponding to subgroup 2.1 of FGE.19, concerns three α,β -unsaturated alicyclic alcohols, *p*-mentha-1,8(10)-dien-9-ol [FL-no: 02.122], 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217], and three esters, santalyl acetate [FL-no: 09.034], santalyl phenylacetate [FL-no: 09.712] and *p*-mentha-1,8(10)-dien-9-yl acetate [FL-no: 09.809], all with the α,β -unsaturation in a side-chain.

The α,β -unsaturated aldehyde and ketone structure is a structural alert for genotoxicity (EFSA, 2008a) and the data on genotoxicity previously available did not rule out this concern for genotoxicity.

For four of these six precursors for α,β -unsaturated alicyclic aldehydes [FL-no: 02.216, 02.217, 09.034 and 09.712], the Panel has identified one structurally related substance in subgroup 1.1.2 of FGE.19 (FGE.201), 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931]. This substance [FL-no: 09.931] from subgroup 1.1.2 is considered representative for the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from FGE.19 subgroup 2.1 (FGE.207) and accordingly, if the genotoxicity

data provided for [FL-no: 09.931] can rule out the genotoxicity concern for this substance, the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] in FGE.19 subgroup 2.1 (FGE.207) can also be cleared for genotoxicity concern. For the remaining alcohol and ester in subgroup 2.1, *p*-mentha-1,8(10)-dien-9-ol [FL-no: 02.122] and *p*-mentha-1,8(10)-dien-9-yl acetate [FL-no: 09.809], the Panel concluded that they could not be represented by the substance [FL-no: 09.931] from subgroup 1.1.2, but the Panel did find that the chemical structure of the two substances [FL-no: 02.122 and 09.809] allowed for a read across between genotoxicity data for the two substances and accordingly the Flavour Industry was requested to submit data for either the alcohol or the ester. The structures of the six substances from FGE.19 subgroup 2.1 (FGE.207) and the one representative substance [FL-no: 09.931], from FGE.19 subgroup 1.1.2, originally evaluated in FGE.201, are shown in Table 2.

Two of the substances from subgroup 2.1 [FL-no: 09.034 and 09.712] have previously been evaluated by the JECFA at their 59th meeting (JECFA, 2002a; JECFA, 2003) and the one representative substance from subgroup 1.1.2 has been evaluated at their 61st meeting (JECFA, 2004a; JECFA, 2004b). A summary of their current evaluation status by the JECFA and the outcome of this consideration is presented in Table 3.

2.2. Representative Substance for Subgroup 2.1

For four [FL-no: 02.216, 02.217, 09.034 and 09.712] of the substances in subgroup 2.1 of FGE.19, the Panel has identified one structurally related substance [FL-no: 09.931] in subgroup 1.1.2 of FGE.19 (FGE.201). This substance from subgroup 1.1.2, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], is considered adequate as representative for the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from subgroup 2.1. Accordingly, the Flavour Industry was requested to submit genotoxicity data for the representative substance from subgroup 1.1.2 in accordance with the test strategy (EFSA, 2008c). The chemical structures of the four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from subgroup 2.1 and the one representative substance [FL-no: 09.931] from subgroup 1.1.2 are shown in Table 1.

Table 1: The Four Substances from Subgroup 2.1 and the Representative Substance of these

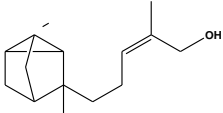
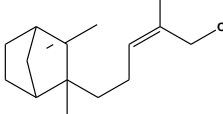
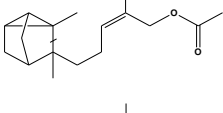
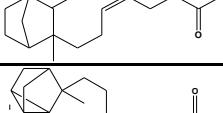
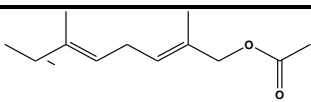
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	EFSA conclusion
02.216	12-beta-Santalen-14-ol		3006 74 77-42-9	[FL-no: 02.216] can be covered by [FL-no: 09.931].
02.217	12-alpha-Santalen-14-ol		3006 74 115-71-9	[FL-no: 02.217] can be covered by [FL-no: 09.931].
09.034 985	Santalyl acetate		3007 224 1323-00-8	[FL-no: 09.034] can be covered by [FL-no: 09.931].
09.712 1022	Santalyl phenylacetate		3008 239 1323-75-7	[FL-no: 09.712] can be covered by [FL-no: 09.931].

Table 1: The Four Substances from Subgroup 2.1 and the Representative Substance of these

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	EFSA conclusion
09.931 1226	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		3886 - 999999-91-4	[FL-no: 09.931] (subgroup 1.1.2) can represent [FL-no: 02.216, 02.217, 09.034 and 09.712].

3. Additional Genotoxicity Data Submitted for Subgroup 2.1 and Subgroup 1.1.2

The Industry has submitted data concerning genotoxicity studies (EFFA, 2012) for one substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] of FGE.19 subgroup 1.1.2 (FGE.201). These data will cover four substances [FL-no: 02.216, 02.217, 09.034 and 09.712] from FGE.19 subgroup 2.1, the present FGE.207.

The new data submitted for 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] covers *in vitro* assays in bacteria and mammalian cell systems.

3.1. *In vitro* Data

3.1.1. Bacterial Reverse Mutation Assay

An Ames assay was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 to assess the mutagenicity of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], both in the absence and in the presence of metabolic activation by S9-mix (from livers of rats induced with Aroclor 1254), in three experiments (King, 2000). An initial experiment was carried out in the absence and presence of S9-mix in the five strains, using final concentrations of 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at 5 - 5000 µg/plate in the presence of S9-mix activation and 5 - 1500 µg/plate in the absence of S9-mix, plus negative (solvent) and positive controls. The standard plate incorporation assay was used. Evidence of toxicity, in terms of a decrease in revertant count, was apparent on all plates treated at 500 µg/plate and above in the absence of S9-mix. In the presence of S9-mix, the test article was toxic at concentrations of 1500 µg/plate and above for strains TA1537 and TA102, and at 5000 µg/plate for strains TA98, TA100, and TA1535. In all cases revertant counts were obtained from at least four different concentrations, and so these data were considered valid for mutation assessment. In the absence of S9-mix activation, no statistically significant increases in revertant numbers were observed in any of the test strains. In the presence of S9-mix activation no statistically significant increases in revertant numbers were observed for strains TA98, TA100, TA1535 or TA1537, but very small increases in revertant numbers were observed in strain TA102 at 15 and 50 µg/plate which, although statistically significant ($p \leq 0.05$), amounted to only 1.17-fold and 1.18-fold increases over background, respectively. Furthermore, no increases were observed at the higher test concentrations of 150 and 500 µg/plate.

In a second confirmatory experiment using the same conditions, no statistically significant increases in revertant numbers were observed at any concentration in any of the strains, either in the presence or absence of S9-mix activation. To further investigate the potential mutagenic effect in strain TA102 in the presence of S9-mix activation, a third experiment was conducted in that strain only. No statistically significant increases in revertant numbers were observed at any concentration tested.

On this basis, the very small increases seen in only a single experiment at the two lower test concentrations in the presence of S9-mix activation in strain TA102 were not reproducible or concentration-related, and were therefore considered to be chance occurrences and not related to treatment with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] (King, 2000). It was concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *S. typhimurium* when tested under

the conditions of this study. These conditions included treatments at concentrations up to either the limit of toxicity or 5000 µg/plate (the maximum recommended concentration, according to current regulatory guidelines), in the absence and in the presence of a rat liver metabolic activation system (S9-mix).

3.1.2. Micronucleus Assays

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] was assayed for the induction of chromosome damage and potential aneugenicity in mammalian cells *in vitro* by examining the effect of compound treatment on the frequency of micronuclei in cultured human peripheral blood lymphocytes (whole blood cultures pooled from two healthy male volunteers in two separate experiments) treated in the absence and presence of a metabolising system (S9-mix) from livers of rats induced with Aroclor 1254 (Whitwell, 2012).

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate was added at 48 hours following culture initiation (stimulation by phytohaemagglutinin) either for 3 hours treatment in the absence or presence of S9-mix plus 21 hours recovery, or for 24 hours treatment in the absence of S9-mix without recovery. Cytochalasin B (6 µg/ml) was added at the start of the 24-hour continuous treatment, or at the start of the 21-hour recovery periods following the 3-hour treatments, in order to block cytokinesis and generate binucleate cells for analysis. It remained in the cultures until they were harvested 24 hours after the start of treatment. A preliminary range-finding experiment had been conducted with and without S9-mix treatment in order to determine the effect of treatment upon Replication Index (RI), which was used as a basis for choosing a range of concentrations to be evaluated in Experiments 1 and 2.

In all of the different treatment conditions and separate experiments, frequencies of micronucleated binucleate cells (MNBN) were normal in negative controls and were significantly increased by treatment with the positive control chemical.

In Experiment 1, all three different treatment conditions described above were investigated. In the first treatment condition, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate was added for 3 hours in the absence of S9-mix at concentrations of 70, 85, 100 or 120 µg/mL along with positive and negative controls, followed by 21 hours recovery. No significant increases in the frequency of MNBN were observed relative to concurrent vehicle controls at any of the concentrations analysed. Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range.

In the second treatment condition, following 24 hours continuous treatment at 20, 40 or 60 µg/mL in the absence of S9-mix without recovery, no increases in the frequency of MNBN cells were obtained that were significantly higher ($p \leq 0.05$) than those observed in concurrent controls. Furthermore, the MNBN cell frequencies in all treated cultures under this treatment condition fell within the 95th percentile of the normal range.

In the third treatment condition, following 3 hours treatment with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at concentrations of 120, 140, 180 or 225 µg/mL in the presence of S9-mix, followed by 21 hours recovery, the frequency of MNBN cells were significantly higher ($p \leq 0.05$) than concurrent controls at the top concentration analysed. This concentration induced a 57 % mean level of cytotoxicity, which is close to the recommended upper limit for this test procedure. Furthermore, increases in the frequency of MNBN cells were only seen in one replicate (A) where only 394 binucleate cells could be analysed for this test concentration, where cytotoxicity actually exceeded 60 %, and where examination of the slides indicated a concentration-related effect on cells without intact cytoplasm. This may have resulted in an underestimation of the cytotoxicity, but it was not observed in the other replicate culture (B).

In Experiment 2, the weak induction of micronuclei that was observed in Experiment 1 in the presence of S9-mix was further investigated. Following treatment for 3 hours followed by 21 hours recovery in

the presence of S9-mix with 2,6-dimethyl-2,5,7-octatriene-1-ol acetate at concentrations of 119.2, 180, 250 or 290 µg/mL, which induced 5 %, 19 %, 39 % and 54 % cytotoxicity, respectively, small but statistically significant ($p \leq 0.05$) increases in MNBN cell frequencies were observed at the lowest and highest concentrations analysed. At the highest concentration analysed only a single replicate culture gave MNBN cell frequencies that exceeded normal historical control values, and it is also noteworthy that the vehicle control frequency was quite low for this particular experiment which might have contributed to the test outcome. Furthermore, additional analysis of spare slides from the replicate cultures at the lowest and highest concentrations analysed resulted in the overall micronucleus frequencies falling within normal ranges. On this basis, the weak statistical significance observed in the first experiment was not reproduced at higher concentrations and similar levels of toxicity, and was therefore not considered to be of biological relevance.

In conclusion, 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] was not considered to demonstrate induction of micronuclei in a robust study that achieved required levels of toxicity (Whitwell, 2012).

4. Conclusion

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] did not induce any biologically significant increases in bacterial mutation when evaluated in an Ames test in the presence and absence of S9 metabolic activation. It did induce weak genotoxic effects in the *in vitro* micronucleus assay in an initial experiment in the presence of S9-mix at the highest concentration only. In a second experiment, although statistically significant increases were observed at the lowest and highest concentrations tested, these increases fell within the historical control range for the testing laboratory, and were not considered to be biologically important. The Panel therefore concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], from subgroup 1.1.2 of FGE.19 (FGE.201), does not give rise to concern with respect to genotoxicity and can accordingly be evaluated through the Procedure. Furthermore, as 2,6-dimethyl-2,5,7-octatriene-1-ol acetate is considered representative for the four precursors for α,β -unsaturated alicyclic aldehydes [FL-no: 02.216, 02.217, 09.034 and 09.712] from subgroup 2.1 of FGE.19 (FGE.207), the genotoxicity concern can also be lifted for these four substances and accordingly they can also be evaluated through the Procedure as well.

SPECIFICATION SUMMARY OF THE SUBSTANCES IN THE FLAVOURING GROUP EVALUATION 207

Table 2: Specification Summary (JECFA, 2002b)

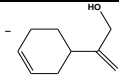
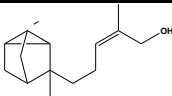
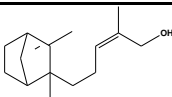
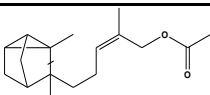
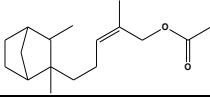
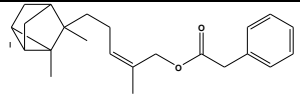
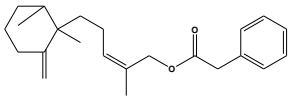
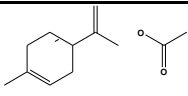
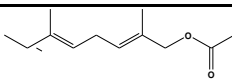
FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)
02.122	p-Mentha-1,8(10)-dien-9-ol		FDA 10239 3269-90-7	Liquid $C_{10}H_{16}O$ 152.24	Practically insoluble or insoluble Freely soluble	104 (1 hPa) MS 95 %	1.494-1.500 0.962-0.968
02.216	12-beta-Santalen-14-ol		3006 74 77-42-9	Liquid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	129 (5.3 hPa) MS 95 %	1.498-1.509 0.965-0.975
02.217	12-alpha-Santalen-14-ol		3006 74 115-71-9	Liquid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	302 MS 95 %	1.498-1.509 0.965-0.975
09.034 985	Santalyl acetate	 	3007 224 1323-00-8	Liquid $C_{17}H_{26}O_2$ 262.40	Insoluble Miscible	20.8 (4 hPa) IR 95 %	1.485-1.493 0.980-0.986
09.712 1022	Santalyl phenylacetate	 	3008 239 1323-75-7	Liquid $C_{23}H_{30}O_2$ 338.49		328 NMR 98 %	1.525-1.576 1.022-1.029
09.809	p-Mentha-1,8(10)-dien-9-yl acetate		10743 15111-97-4	Liquid $C_{12}H_{18}O_2$ 194.27	Practically insoluble or insoluble Freely soluble	218 MS 95 %	1.473-1.479 0.971-0.977
09.931 1226	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		3886 999999-91-4	Liquid $C_{12}H_{18}O_2$ 194.28	Insoluble Soluble	70 (3 hPa) MS	1.490-1.500 0.937-0.947

Table 2: Specification Summary (JECFA, 2002b)

FL-no	EU Register name	Structural formula	FEMA no	Phys.form	Solubility 1)	Boiling point, °C 3)	Refrac. Index 4)
JECFA-no			CoE no	Mol.formula	Solubility in ethanol 2)	Melting point, °C	Spec.gravity 5)
			CAS no	Mol.weight		ID test	
						Assay minimum	
						96 %	

- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95 % ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.
- 6) Stereoisomeric composition not specified.

CURRENT SAFETY EVALUATION STATUS APPLYING THE PROCEDURE (BASED ON THE MSDI APPROACH)

Table 3: Summary of Safety Evaluation of the JECFA Substances in FGE.207 (JECFA, 2002a)

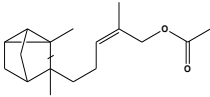
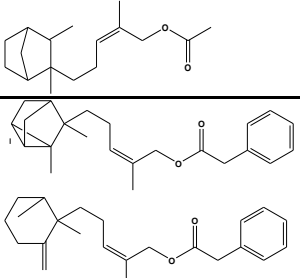
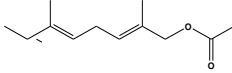
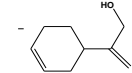
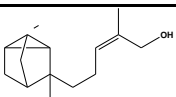
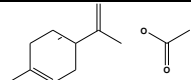
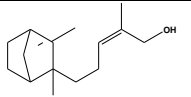
FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (genotoxicity)
09.034 985	Santalyl acetate		ND 0.01	Class I A3: Intake below threshold	4)	Evaluated in FGE.207, no genotoxicity concern, to be evaluated through the Procedure.
09.712 1022	Santalyl phenylacetate		ND 1	Class I A3: Intake below threshold	4)	Evaluated in FGE.207, no genotoxicity concern, to be evaluated through the Procedure.
09.931 1226	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		1.2 7.7	Class I A3: Intake below threshold	4)	Evaluated in FGE.201Rev1, additional genotoxicity data required. New data evaluated in FGE.207, no genotoxicity concern, to be evaluated through the Procedure.
02.122	p-Mentha-1,8(10)-dien-9-ol		0.012	Class I No evaluation	Not evaluated by the JECFA	Evaluated in FGE.207, additional genotoxicity data required
02.216	12-beta-Santalén-14-ol		0.085	Class I No evaluation	Not evaluated by the JECFA	Evaluated in FGE.207, no genotoxicity concern, to be evaluated through the Procedure.

Table 3: Summary of Safety Evaluation of the JECFA Substances in FGE.207 (JECFA, 2002a)

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI ($\mu\text{g/capita/day}$)	Class 2) Evaluation procedure path 3)	JECFA Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (genotoxicity)
09.809	p-Mentha-1,8(10)-dien-9-yl acetate		0.012	Class I No evaluation	Not evaluated by the JECFA	Evaluated in FGE.207, additional genotoxicity data required.
02.217	12-alpha-Santalen-14-ol		0.11	No evaluation	Not evaluated by the JECFA	Evaluated in FGE.207, no genotoxicity concern, to be evaluated through the Procedure.

1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = $\mu\text{g/capita/day}$.

2) Thresholds of concern: Class I = 1800 $\mu\text{g/person/day}$, Class II = 540 $\mu\text{g/person/day}$, Class III = 90 $\mu\text{g/person/day}$.

3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

4) No safety concern based on intake calculated by the MSDI approach of the named compound.

5) Data must be available on the substance or closely related substances to perform a safety evaluation.

ND: not determined

GENOTOXICITY (*IN VITRO*)

Table 4: Summary of Additionally Genotoxicity Data for [FL-no: 09.931] of Subgroup 1.1.2

Chemical Name [FL-no:]	Test System <i>in vitro</i>	Test Object	Concentrations of Substance and Test Conditions	Result	Reference	Comments
2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [09.931]	Reverse Mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA102	5 - 1500 µg/plate [1,3]; 5 - 5000 µg/plate [2,3]	Negative [1,3]; Equivocal [2,3]	(King, 2000)	Reliable without restriction. GLP study in compliance with OECD Guideline 471. A small increase in TA102 revertant numbers was seen at 15 and 50 µg/plate in the presence of S9-mix, but not at higher concentrations.
		<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537 and TA102	5 - 1500 µg/plate [1,3]; 5 - 5000 µg/plate [2,3]	Negative [1,3]; Negative [2,3]		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the second experiment.
		<i>S. typhimurium</i> TA102	5 - 1500 µg/plate [2,3]	Negative		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the third experiment.
	Micronucleus Assay	Human peripheral blood lymphocytes (Male Donors)	70 - 120 µg/ml [1,4]; 120 - 225 µg/mL [2,4]; 20 - 60 µg/mL [1,5]; 119.2 - 290 µg/mL [2,4]	Weak positive +S9; Re-test within normal values	(Whitwell, 2012)	Reliable without restriction. GLP study in compliance with OECD Guideline 487. Weak evidence of inducing micronuclei in the presence of S9-mix in a first experiment (increases only in one culture). A re-test under the same conditions and using a higher top concentration resulted in MNBN frequencies within the historical negative control range at 95 th percentile, but were statistically significant due to low vehicle control values.

[1] Without S9-mix metabolic activation.

[2] With S9-mix metabolic activation.

[3] Plate incorporation method.

[4] 3-hour incubation with 21-hour recovery period.

[5] 24-hour incubation with no recovery period.

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ABBREVIATIONS

CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CoE	Council of Europe
EFSA	The European Food Safety Authority
EU	European Union
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
ID	Identity
IR	Infrared spectroscopy
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
MNBN	MicroNucleated BiNucleate cells
MS	Mass spectra
NMR	Nuclear Magnetic Resonance
No	Number
OECD	Organisation for Economic Co-operation and Development
(Q)SAR	(Quantitative) Structure Activity Relationship
RI	Replication Index
WHO	World Health Organisation